

# High-Speed Video Analysis of the Escape Responses of the Copepod *Acartia tonsa* to Shadows

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**Abstract.** The copepod *Acartia tonsa* exhibits a vigorous escape jump in response to rapid decreases in light intensity, such as those produced by the shadow of an object passing above it. In the laboratory, decreases in light intensity were produced using a fiber optic lamp and an electronic shutter to abruptly either nearly eliminate visible light or reduce light intensity to a constant proportion of its original intensity. The escape responses of *A. tonsa* to these rapid decreases in visible light were recorded on high-speed video using infrared illumination. The speed, acceleration, and direction of movement of the escape response were quantified from videotape by using automated motion analysis techniques. *A. tonsa* typically responds to decreases in light intensity with an escape jump comprising an initial reorientation followed by multiple power strokes of the swimming legs. These escape jumps can result in maximum speeds of over  $800 \text{ mm s}^{-1}$  and maximum accelerations of over  $200 \text{ m s}^{-2}$ . In *A. tonsa*, photically stimulated escape responses differ from hydrodynamically stimulated responses mainly in the longer latencies of photically stimulated responses and in the increased number of power strokes, even when the stimulus is near threshold; these factors result in longer escape jumps covering greater distances. The latency of responses of *A. tonsa* to this photic stimulus ranged from a minimum of about 30 ms to a maximum of more than 150 ms, compared to about 4 ms for hydrodynamically stimulated escape jumps. Average response latency decreased with increasing light intensity or increasing proportion of light eliminated. Little change was

observed in the vigor of the escape response to rapid decreases in visible light over a wide range of adaptation intensities.

## Introduction

Planktonic copepods are an important link in marine food webs between microplankton and higher trophic levels. Copepods are well known for their vigorous escape responses (*e.g.*, Singarajah, 1969; Fields and Yen, 1997), which play an important role in predator avoidance (*e.g.*, Drenner *et al.*, 1978; Viitasalo *et al.*, 1998). These escape responses can be elicited by both hydrodynamic (Hartline *et al.*, 1999; Kiorboe *et al.*, 1999; Lenz and Hartline, 1999) and photic stimuli (Buskey *et al.*, 1986, 1987). Despite the scarcity of direct evidence that chemosensory stimuli, by themselves, can produce vigorous escape responses in calanoid copepods, there is evidence that such stimuli can cause copepods to exhibit changes in swimming activity (*e.g.*, Katona, 1973; Buskey, 1984). Predator-specific chemicals have also been shown to alter vertical migration behavior in freshwater zooplankton (*e.g.*, Tjossem, 1990; Ringelberg, 1991) and marine crab larvae (Forward and Rittschof, 2000), but similar effects are yet to be demonstrated in marine copepods (*e.g.*, Bollens *et al.*, 1994).

Light has long been known to have an important effect on the behavior of planktonic organisms, with much research emphasizing the effects of light on vertical migration (reviewed in Forward, 1988). Photophobic responses of copepods are thought to play a role in planktonic predator-prey interactions—both in terms of a predator-deterrent role of bioluminescence in dark-adapted copepods (Buskey and Swift, 1983, 1985) and in terms of a predator-avoidance role for copepods exposed to shadows in light-adapted copepods (Buskey *et al.*, 1986). Alterations in behavior of planktonic

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organisms in response to decreases in light intensity have been demonstrated in neritic calanoid copepods (Buskey *et al.*, 1987) and crab larvae (Forward, 1977).

Recent studies have used strain gauges and high-speed video to provide the high temporal resolution necessary to describe, in detail, the kinetics of the escape responses of both tethered (Lenz and Hartline, 1999; Hartline *et al.*, 1999) and free-swimming (Buskey *et al.*, 2002) calanoid copepods to hydromechanical disturbances. These studies demonstrate that copepod response latencies (down to 2 ms) are among the fastest ever recorded. This rapidity is in part due to the myelination of the nerves of some calanoid copepods (Davis *et al.*, 1999), although extremely fast responses have also been found in *Acartia tonsa* (4 ms), which does not possess myelinated nerves (Buskey *et al.*, 2002). In this study, we use high-speed video recording and computerized motion analysis techniques to describe the kinetics of the escape responses of the calanoid copepod *A. tonsa* to photic stimuli, and we compare the results to those of previous studies that examined the escape behavior of this species in response to hydrodynamic stimuli.

### Materials and Methods

During March and April 2001, live zooplankton were collected from the University of Texas Marine Science Institute pier located in the Aransas Ship Channel connecting the Gulf of Mexico with Aransas and Corpus Christi bays. A 0.5-m diameter, 153- $\mu\text{m}$ -mesh plankton net was allowed to stream with the tidal current for 2–3 min. The contents of the cod end were diluted into a plastic bucket containing whole seawater and returned to the laboratory for sorting. The sample was examined under a dissecting microscope and adult male and female *Acartia tonsa* were removed using a wide-bore pipette. Twenty adults of a single sex were then placed in small, clear acrylic plastic aquaria (60  $\times$  30  $\times$  60 mm) containing filtered seawater. Each set of copepods was used in only one experiment. Before an experiment, the copepods to be used were allowed to adapt to a specific light intensity (100, 10, 1, 0.3 or 0.1  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) for at least 1 h at near-ambient temperatures (19  $\pm$  1  $^{\circ}\text{C}$  for complete shadow experiments, 22  $^{\circ}\text{C}$  for partial shadow experiments).

Experiments were performed in a darkroom, and red-filtered flashlights were used for viewing equipment and taking notes, since *A. tonsa* has a very low visual sensitivity to red light (Stearns and Forward, 1984a). For experiments testing the response of *A. tonsa* to complete shadows (adaptation light intensity reduced by 100%), a Dolan Jenner model 3100 Fiber Lite illuminator was used as the light source for the stimulus. A single light guide was oriented vertically above the aquarium containing the copepods, producing a beam of about 2.5-cm diameter on a sheet of opal glass resting on top of the aquarium. Light intensity

was adjusted to produce a nominal light intensity of 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  as measured in air near the center of the aquarium; light intensity was measured using a LICOR LI-250 light meter with a LI-109A quantum sensor. The light was cut off using an electronic shutter for a period of 500 ms (Vincent Associates Uniblitz SD-10 shutter driver timer and model 255 shutter). Light intensities of 10, 1, 0.3, and 0.1  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  were produced using a combination of neutral-density filters placed above the shutter at the tip of the light probe. The copepods were isolated from vibrational stimuli using a Newport bench-top vibration-isolating table.

In experiments where *A. tonsa* was subjected to partial reduction in light intensity, light was provided by a Dolan Jenner Model PL-180 quartz-halogen illuminator with dual branch light guides. The light beam from each branch of the light guide was directed from opposite sides at an angle of about 30 $^{\circ}$  from vertical at the same area of opal glass directly over the small aquarium containing the copepods. One branch was fitted with the electronic Uniblitz shutter to cut off a fraction of the light striking the aquarium. The relative contribution of the light guides to total illumination of the chamber was modified using neutral-density filters. Only adult female copepods were used in studies of escape responses to partial reduction of light intensity.

In an experimental run, each group of copepods was allowed to further adapt to the new experimental setup for a minimum of 30 min after being moved. The position of copepods in the field of view was observed on the television monitor, and the shutter was actuated when at least 2 copepods could be observed. After each group of copepods was stimulated, the high-speed video would be played back at slow speed (1 frame  $\text{s}^{-1}$ ) to record the number of copepods responding to the stimulus and the latency of the response. Each group of copepods was subjected to decreases in light intensity several times until a minimum of 20 copepods had been observed. These experiments were repeated with at least six different groups of copepods under each set of light-adaptation conditions.

Images of copepod escape responses were recorded at 1000 frames per second using a high-speed video camera (Kodak Motion Corder Analyzer Model SR-3000). The camera was equipped with a 50-mm f1.4 Nikon Nikkor lens, and viewed an area of about 25  $\times$  30 mm. Additional lighting for recording copepod images during the absence or decrease of visible light during shadows was provided by a dark-field array of infrared-light-emitting diodes. The high-speed video camera recorded 2184 frames of video at 1000 frames per second. A signal from the electronic shutter was used to trigger the camera to capture half the video frames before and half after the decrease in light intensity. These frames were then played back at 30 frames per second and recorded on a Panasonic AG 6300 videocassette recorder.

These high-speed video records were then reviewed to

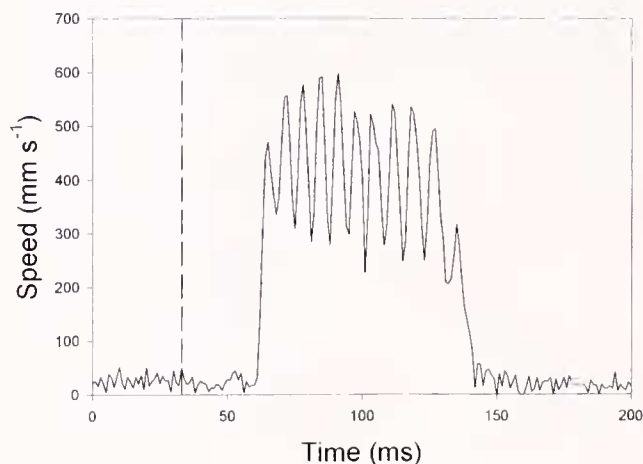
choose escape paths for computerized motion analysis. Analysis of the behavioral parameters associated with the complete escape response of *Acartia tonsa* was hindered by the vigor of the response. To have the image of the copepods large enough to be automatically tracked by our motion analysis system, the field of view was limited to about  $25 \times 30$  mm, and some copepods swam out of the field of view during their escape response. We attempted to quantify behavioral parameters for 20 escape responses for each set of conditions. We chose escape responses in which the copepod's initial position could be observed, and whose trajectories covered a minimum of one-third of the screen (tracked for at least 1 cm). Because escape behaviors were recorded in two dimensions, only trajectories perpendicular to the recording camera were accurately recorded. To minimize errors through analysis of paths not perpendicular to the camera, the aspect ratios of copepod images were measured at the beginning, middle, and near the end of their escape jumps (Buskey *et al.*, 2002). Aspect ratios greater than 3 indicated that a copepod was nearly perpendicular to the line of view of the video camera. Escape responses with an aspect ratio less than 2.5 during part of their trajectory were not used for motion analysis. Since only 20 escape responses were quantified out of the more than 120 copepods observed, instances in which escape responses were recorded from the same copepod should be rare, if they occurred at all.

Swimming behavior of the copepods was quantified from videotapes using an Expertvision Cell-Trak video-computer motion-analysis system. High-speed video recorded at 1000 frames per second was transferred to videotape at 30 frames per second. These videotaped images of the copepods were digitized using the Motion Analysis VP-110 video-to-digital processor, and digitized outlines of the copepods were sent to a host computer at a rate of 30 frames per second. These digitized images were processed using Cell-Trak software to calculate the swimming speeds ( $\text{mm s}^{-1}$ ), acceleration ( $\text{m s}^{-2}$ ), and number of swimming leg thrusts per jump of the copepod's paths of travel. Jump latencies (time from stimulus to response) were determined by reviewing the videotapes frame-by-frame for a minimum of 200 ms. High-speed video analysis revealed an average delay of 6-ms between the signal sent to start the video sequence and the closing of the electronic shutter. Response latencies were corrected for this delay in stimulus onset. A copepod occasionally performed a single spontaneous jump during the 200-ms period after the decrease in light intensity. Spontaneous jumps consist of a single thrust of the swimming legs with lower peak speeds than escape jumps (Buskey *et al.*, 2002). These occurred both before and after the decrease in light intensity and were not counted as photic escape responses.

## Results

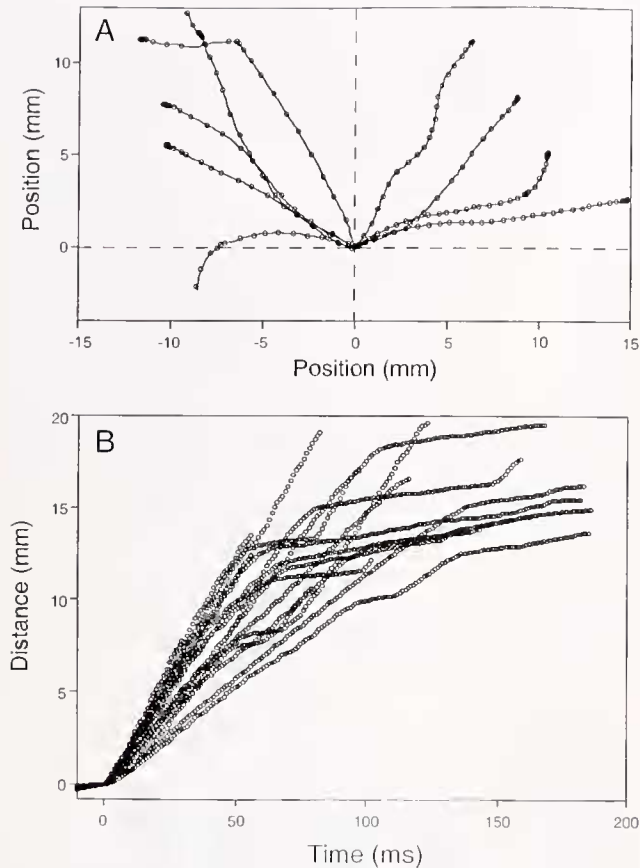
*Acartia tonsa* responds to a rapid decrease in light intensity with a series of vigorous thrusts of the swimming legs. An example of a record of changes in swimming speed over time for the response of an adult female adapted to  $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and subjected to a complete reduction in visible light is shown in Figure 1. In this example, the dashed vertical line represents the time at which light intensity decreased; the response was initiated 32 ms later as a series of 11 thrusts of the swimming legs. Although the action was not apparent in the swimming speed record, examination of high-speed video revealed a rapid reorientation of the body axis by about 40 degrees during the 2-ms period preceding the first escape thrust. Maximum speeds during these swimming thrusts ranged between 400 and 600  $\text{mm s}^{-1}$ , with minimum speeds between thrusts decreasing to between 200 and 350  $\text{mm s}^{-1}$ . The duration of each thrust of the swimming legs is about 8 ms (time from minimum speed at the beginning of one thrust to minimum speed at the beginning of the next thrust). The entire response lasted about 80 ms and displaced the copepod by about 31 mm. Examples of escape trajectories during photically stimulated escape jumps are shown in Figure 2A. These escape jumps, plotted from a common starting point, typically displace the copepod 10 to 15 mm from its original location. The range of responses observed, in terms of distance moved over time, ranges from 5 to 15 mm within the first 50 ms of the escape jump (Fig. 2B).

For complete reduction in light intensity, the proportion of copepods exhibiting an escape response varied with the light adaptation intensity. For adult male copepods, adapta-



**Figure 1.** Example of a record of swimming speed over time for the photically stimulated escape response of an adult female specimen of *Acartia tonsa*. This copepod was adapted to a light intensity of  $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and subjected to a 100% decrease in light intensity. The vertical dashed line represents the beginning of the decrease in light intensity.





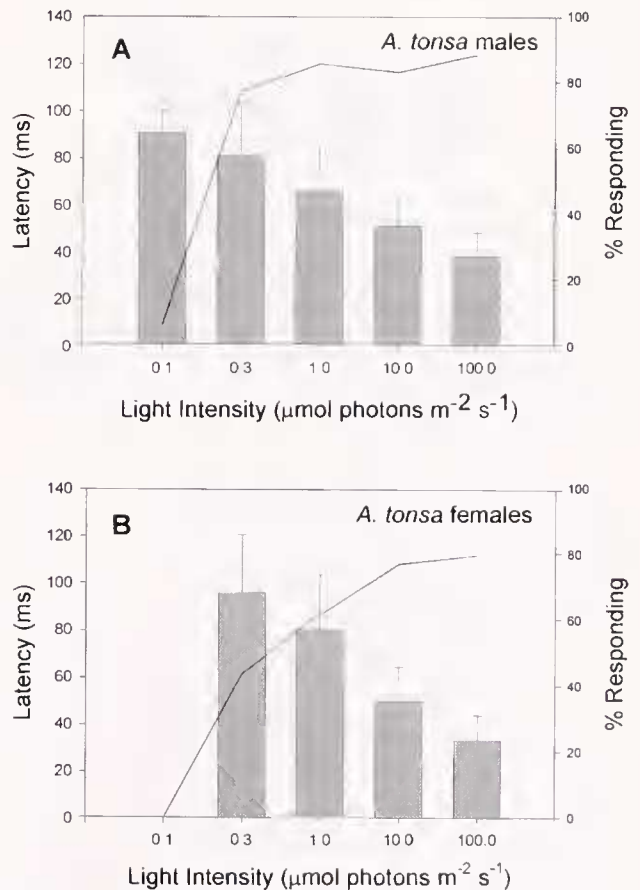
**Figure 2.** Displacement trajectories in the vertical plane plotted from a common starting point for escape responses to rapid decreases in light intensity of adult female specimens of *Acartia tonsa* (A). Distance from original position plotted against time (B).

tion intensities of  $0.3 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and higher resulted in a response by about 80% of the copepods (Fig. 3A). For an adaptation intensity of  $0.1 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , the response rate dropped rapidly to less than 10%. For adult females of *A. tonsa* the change in response rate with light adaptation intensity changed more gradually (Fig. 3B). At both 10 and  $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , response rate was similar to that of males at the same intensity, about 80%. However, the response rate dropped to about 60% at  $1.0 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and to less than 50% at  $0.3 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . None of the females responded at an adaptation intensity of  $0.1 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Therefore, the threshold light intensity for stimulating escape responses with complete shadows is between  $0.1$  and  $0.3 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  for both adult males and females of *A. tonsa*.

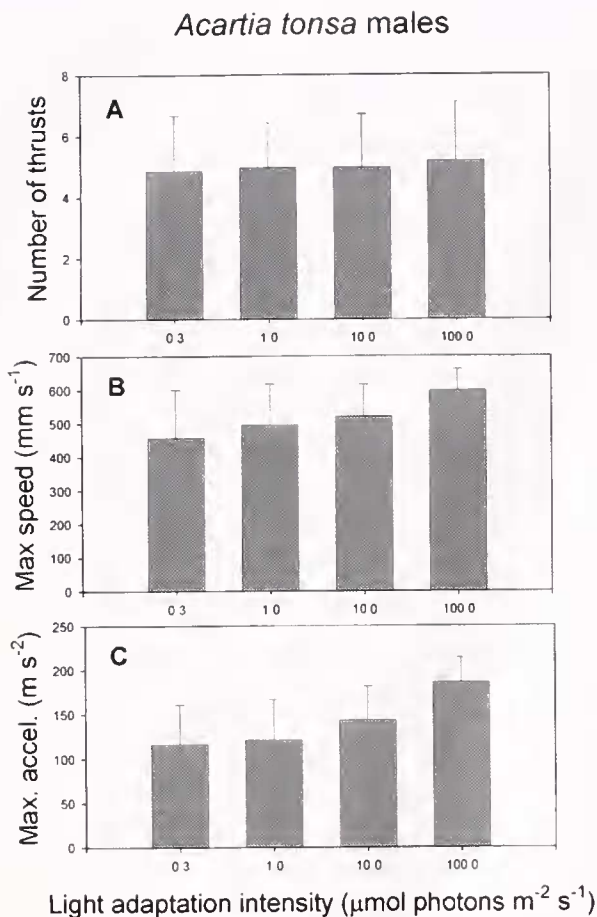
For complete reduction in light intensity, the response latency between the initiation of the change in light intensity and the initiation of the first escape movement increases as the light adaptation intensity decreases (Fig. 3). For adult males, the mean response latencies progressively changed

from 38 ms at  $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  to 90 ms at  $0.1 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . A Kruskal-Wallis one-way ANOVA on ranks indicated a significant difference in response latencies at different adaptation intensities ( $P < 0.001$ ). Dunn's method for pairwise comparisons revealed that all pairs of groups were significantly different from one another ( $P < 0.05$ ). For adult females, the mean response latencies changed from 33 ms at  $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  to 96 ms at  $0.3 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . A Kruskal-Wallis one-way ANOVA on ranks again indicated a significant difference in response latencies at different adaptation intensities ( $P < 0.001$ ). Pairwise comparisons using Dunn's method indicated that latencies were significantly different from one another ( $P < 0.05$ ) except for comparison of adaptation intensities of 0.3 and  $1 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ .

Although the response latencies changed for adult males of *A. tonsa* over the range of light adaptation intensities from 0.3 to  $10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , there was no evidence that the vigor of their escape behavior changed sig-



**Figure 3.** The percentage of copepods responding to complete shadows (solid line) for copepods adapted to a range in light intensities, and the mean latency between escape responses and initiation of the change in light intensity. Error bars represent 1 standard deviation. Responses of adult male (A) and adult female (B) specimens of *Acartia tonsa*.

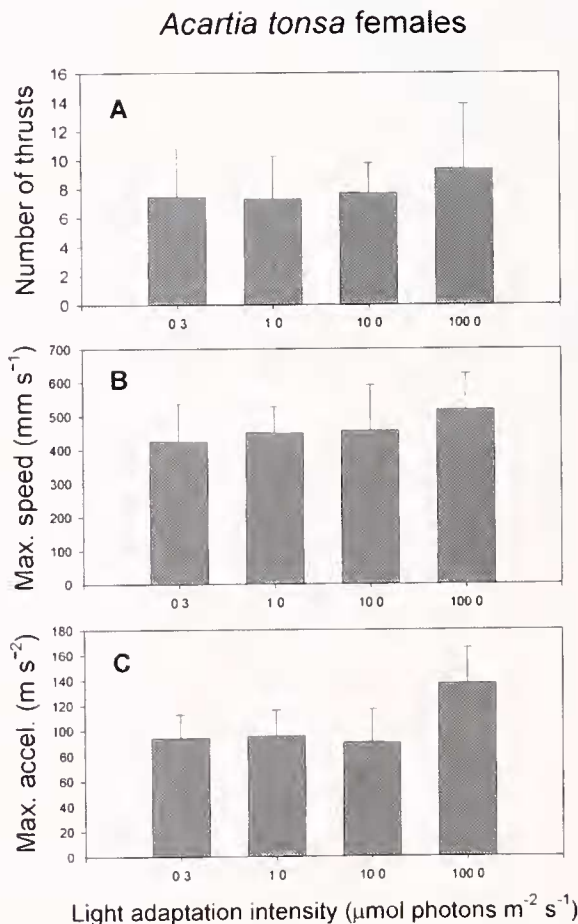


**Figure 4.** Characteristics of escape responses for male specimens of *Acartia tonsa* exposed to complete shadows, including average number of thrusts per escape response (A), maximum speed ( $\text{mm s}^{-1}$ ) achieved during escape (B), and maximum acceleration ( $\text{m s}^{-2}$ ) during escape (C). Copepods were adapted to light intensities of 0.3, 1, 10, or 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Error bars represent 1 standard deviation.

nificantly until an adaptation intensity of 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  was applied (Fig. 4). Although no significant change in the number of swimming leg thrusts in each escape response was observed, there was a significant change in average speed ( $P < 0.001$ , one-way ANOVA, data not shown), maximum speed ( $P = 0.001$ , Kruskal-Wallis one-way ANOVA on ranks), and maximum acceleration ( $P < 0.001$ , one-way ANOVA). Pairwise comparisons revealed that average speeds and maximum accelerations of adult males during escape responses were significantly different at an adaptation intensity of 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  from those at each other intensity tested (Tukey test,  $P < 0.05$ ). For maximum speed, adult male copepods adapted to 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  were significantly different only from those at 1.0 and 0.3  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  adaptation intensities (Dunn's method,  $P < 0.05$ ).

Adult females of *A. tonsa* tested over a range of adapta-

tion intensities from 0.3 to 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  also showed some differences in escape behaviors at the highest adaptation intensity (Fig. 5). There was no significant difference in the number of thrusts of the swimming legs in each escape jump (one-way ANOVA,  $P = 0.057$ ) or in average speed of escape responses over this range of adaptation intensities (one-way ANOVA,  $P = 0.170$ , data not shown). For maximum speed during escape, there was a significant difference between escape responses of copepods adapted to different light intensities (Kruskal-Wallis one-way ANOVA on ranks,  $P = 0.04$ ). Dunn's method for pairwise comparison for all pairs revealed that only the maximum speeds for escape responses of copepods adapted to 0.3 and 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  were significantly different ( $P < 0.05$ ). For maximum acceleration achieved during escape responses, there was also a significant difference between copepods adapted to different light intensities (one-way ANOVA,  $P < 0.001$ ). Pairwise comparison us-



**Figure 5.** Characteristics of escape responses for female specimens of *Acartia tonsa* exposed to complete shadows, including average number of thrusts per escape response (A), maximum speed ( $\text{mm s}^{-1}$ ) achieved during escape (B), and maximum acceleration ( $\text{m s}^{-2}$ ) during escape (C). Copepods were adapted to light intensities of 0.3, 1, 10 or 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Error bars represent 1 standard deviation.

Table 1

Summary of escape response parameters for adult females and males of *Acartia tonsa* exposed to photic stimuli

Parameter	Females	Males	P
Latency (ms)	68.2 (1.8; 29–159)	62.2 (1.3; 35–138)	<0.001†
Initial turn (deg)	41.3 (3.3; 5–103)	74.1 (4.6; 12–165)	<0.001*
Jump speed (mm s <sup>-1</sup> )	256 (10; 125–476)	293 (12; 83–383)	0.453
Maximum speed (mm s <sup>-1</sup> )	446 (15; 235–827)	494 (16; 214–727)	0.002*
Max. acceleration (m s <sup>-2</sup> )	93.3 (10.1; 54–156)	128 (6.0; 41–211)	<0.001*
Number of thrusts	7.5 (0.4; 3–15)	4.9 (0.2; 2–9)	<0.001*
Minimum speed between thrusts (mm s <sup>-1</sup> )	130 (2.2; 65–230)	147 (3.2; 65–256)	<0.001*
Thrust duration (ms)	8.8 (0.1; 4–13)	8.1 (0.1; 6–14)	<0.001*
Jump duration (ms)	74.6 (3.6; 33–136)	44.5 (1.8; 16–70)	<0.001*
Distance jumped (mm)	12.5 (0.6; 5.3–25.5)	8.6 (0.5; 3.4–15.1)	<0.001*

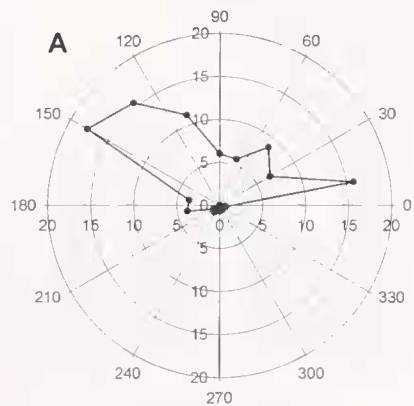
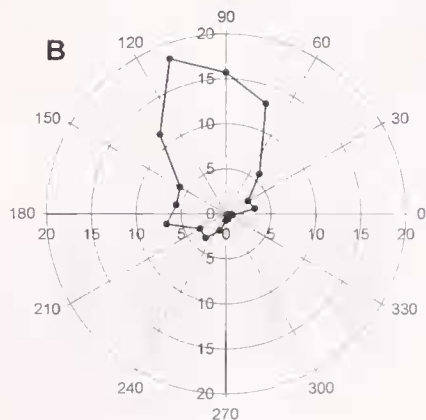
Data are pooled for jumps with adaptation intensities of 10, 1, and 0.3  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Means are based on 54 escape jumps for females or 53 escape jumps for males. Standard error and range are given in parentheses. Probability (*P*) is based on the Mann-Whitney sum rank test. An asterisk \* indicates a significant difference ( $\alpha = 0.05$ ).

ing Tukey's test revealed that copepods adapted to 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  had significantly different maximum accelerations from each of the other adaptation intensities ( $P < 0.05$ ).

Since no significant differences were found between between escape response parameters for copepods adapted to 10, 1, or 0.3  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , results from these three intensities were pooled for comparisons between male and female escape response parameters. Females had significantly longer response latencies than males, but significantly smaller initial turns (Table 1). Although there were no significant differences in the average escape speed of males and females, males exhibited significantly higher maximum speeds and maximum accelerations than females. Females also performed significantly more thrusts per escape jump than did males: the time between thrusts was longer and the minimum speed between thrusts was slower for females compared to males (Table 1). Overall duration of the escape jump was significantly longer for females, and the distance jumped was significantly farther for females than for males.

There was also a difference in the dominant directionality of the escape responses between copepods adapted to low light intensities (1  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and higher adaptation intensities (100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Analysis of the direction of travel of the copepods during their high-speed escape responses reveals that for copepods adapted to low light intensities, the dominant swimming direction in response to a complete shadow was upward (Fig. 6B), whereas copepods adapted to higher light intensities showed a larger proportion of lateral swimming during escape responses (Fig. 6A).

For copepods exposed to only partial reductions in light intensity, which should correspond to light change more similar to a natural shadow in nature, the proportion of copepods responding to these changes in light intensity

100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  adaptation1  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  adaptation

**Figure 6.** Polar plot of direction of travel in the vertical plane during escape responses of adult female specimens of *Acartia tonsa* adapted to 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  light (A) or 1  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (B). Plots represent the percentage of time traveled in each direction during escape behaviors. The coordinate system is arbitrary, with 0 degrees representing motion to the right, 90 degrees representing motion upward, and so forth.



increased as the percent change in light intensity was increased (Fig. 7). For adult females adapted to a low light intensity ( $10 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ ), the proportion of copepods responding to light reductions dropped sharply from near 80% for a 100% reduction in light intensity to less than 10% for a 21% reduction in light intensity. For copepods adapted to higher light intensity ( $100 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ ), the proportion of copepods responding to reductions in light intensity declined more gradually from a response of 80% to a complete shadow to a response of less than 20% to a 21% shadow (Fig. 7). Response latencies did not change with percent light reduction in a systematic way. For adult females adapted to  $10 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ , there were significant differences in latency between copepods responding to 100%, 45%, and 28% reduction in light intensity (Kruskal-Wallis one-way ANOVA on ranks,  $P = 0.001$ ). Too few copepods responded to a 21% reduction in light intensity to compare their latencies. Pairwise compar-

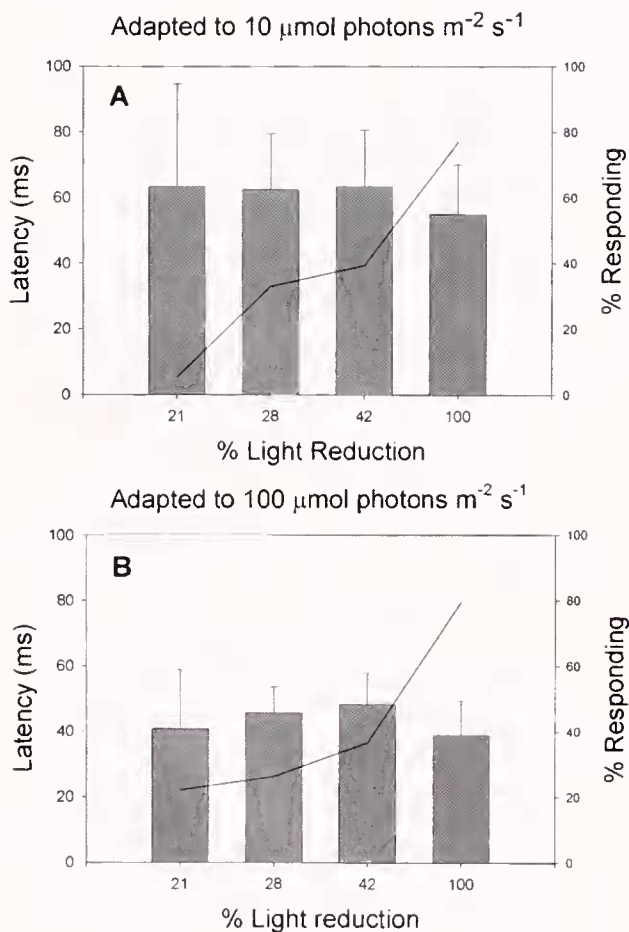
ison to latencies at 100% reduction in light intensity showed that both groups differed significantly from this control group (Dunn's method,  $P < 0.05$ ). For copepods adapted to  $100 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ , there were also significant differences in response latencies between groups (Kruskal-Wallis one-way ANOVA on ranks,  $P < 0.001$ ), and latencies for 45% and 28% reductions in light intensity were significantly different from control latencies for complete shadows, but there was no difference for 21% reductions (Dunn's method).

## Discussion

Calanoid copepods are consumed by a wide range of pelagic predators including other copepods, chaetognaths, jellyfish, fish, and whales. Their primary sensory system for direct detection of predators is often considered to be mechanoreception; copepods are extraordinarily sensitive to small hydromechanical disturbances (Kiorboe and Visser, 1999; Lenz and Hartline, 1999). Photoreception may also play a role in predator avoidance by copepods. Most calanoid copepods, including *Acartia tonsa*, possess only a simple nauplius eye consisting of paired dorsal ocelli and a single ventral ocellus (Elofsson, 1966). These simple photoreceptors are not capable of image formation, but photoreception is thought to regulate the timing and extent of vertical migration in many copepod species (*e.g.*, Stearns and Forward, 1984b; reviewed in Forward, 1988) which indirectly helps copepods avoid attack by visual predators by remaining in low light environments during daylight hours.

Calanoid copepods may use photoreception to avoid direct attacks by predators through photophobic responses to rapid increases or decreases in light intensity (Buskey *et al.*, 1987). A commonly suggested function of the bioluminescence of dinoflagellates is as a deterrent against nocturnal predation (Tett and Kelly, 1973; Porter and Porter, 1979; Morin, 1983). Dark-adapted copepods exhibit strong escape responses to both natural and simulated dinoflagellate bioluminescence (Buskey *et al.*, 1983; Buskey and Swift, 1983, 1985). Dinoflagellate bioluminescence stimulated during the feeding of copepods may attract visual predators on the copepods through a "burglar alarm" effect (Burkenroad, 1943; Mensinger and Case, 1992; Abrahams and Townsend, 1993). Light-adapted copepods exhibit similar escape responses when exposed to rapid decreases in light intensity (Buskey *et al.*, 1986, 1987). These shadow responses might help copepods avoid "hydrocryptic" predators such as ctenophores and enidarian medusae that produce only small hydrodynamic disturbances but cast shadows during the day as they descend in the water column.

To provide an effective defense against shadow-casting predators, it might seem that laterally oriented escape trajectories would be more effective than vertically oriented



**Figure 7.** The percentage of adult female specimens of *Acartia tonsa* responding to shadows (solid line) with constant fractions of light reduction and the mean latency between escape responses and initiation of change in light intensity. Error bars represent 1 standard deviation. Copepods adapted to  $10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (A), and copepods adapted to  $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (B).

responses, which might propel the copepod toward the predator. In this study, copepods adapted to low light intensities more often showed vertically oriented escape paths, while those adapted to higher light intensities more often moved laterally (Fig. 6). In a previous study of shadow responses of *Acartia tonsa* from Narragansett Bay, Rhode Island, copepods adapted to these lower intensities showed laterally directed escape responses (Buskey *et al.*, 1986). The reasons for the regional differences in behavioral responses of *A. tonsa* are uncertain. Coastal bays in Texas are generally shallower than Narragansett Bay and have weaker tidal currents (Hess and White, 1974; Armstrong, 1987). Members of this species are known to be strong vertical migrators (Stearns and Forward 1984b); in shallow subtropical bays they are closely associated with the bottom during the day (Stubblefield *et al.*, 1984; Buskey, 1993), rather than being suspended in the water column as they are in deeper environments with stronger currents. As a defense against shadow-generating benthic predators such as bottom-feeding fish, vertically directed escape responses might be effective adaptations for copepods closely associated with the bottom during the day.

Previous studies have used the same high-speed video analysis to examine hydrodynamically stimulated escape responses in *A. tonsa* (Buskey *et al.*, 2002). One of the salient differences we found between the photically and mechanically triggered escape behaviors in this species was the substantially larger minimum reaction time of the former (29 ms vs. 3 ms to first movement; Tables 1, 2). The shortest photically triggered behavioral responses we have found in the invertebrate literature is about 30 ms for the startle response (mesothoracic leg thrust) to sudden light decreases in *Drosophila melanogaster* (review: Wyman *et al.*, 1984). Larger animals are somewhat slower: 450 ms for

the withdrawal reflex of leech (Laverack, 1969); 50 ms for the photic startle (flash) response of squid (Neumeister *et al.*, 2000); and 30 ms for the photophobic fast-start response to sudden darkening in small fish (Hartline, unpubl. data). In contrast, the reaction latencies to mechanical stimulation of several species is considerably shorter: 40 ms for leech (Laverack, 1969); 11 ms for response to air puff in slowly walking cockroach (Camhi and Nolen, 1981); and 5 ms for the Mauthner-mediated fast-start in adult zebra fish (Eaton *et al.*, 1977). Part of the reason for the difference between the two modalities is in the intrinsic speed of the sensory mechanism mediating the reaction. Mechanoreceptors in a wide variety of species employ direct mechanical links to transmit movement of the receptive surface (*e.g.*, a seta) directly to the ion channel in the membrane of the receptor cell, thus reducing delays to onset of receptor potentials to tens of microseconds (*e.g.*, Thurm, 1965; review: French, 1992). There is no need for time-consuming amplification beyond the initial molecular transduction event. Photic signals, on the other hand, involve an extensive biochemical cascade, including amplification of the original molecular event (photoisomerization of a rhodopsin-like compound) and diffusion of reactants to ionic channel sites in receptor membranes (review: Goldsmith, 1991). This takes time. Minimum latencies to onset of electrical changes are measured in milliseconds—10–20 ms in slow arthropod eyes, including most crustaceans; 3–6 ms in fast insect eyes (Bullock and Horridge, 1965); 7 ms in cuttlefish (Weeks and Duncan, 1974); 15–50 ms in cold-blooded vertebrates (Tomita, 1970, review and citations); 2 ms in monkey [at 38 °C], (Brown *et al.*, 1965). Thus the photic modality is an inherently slow one, and the faster mechanical one has some clear advantages for animal survival.

A second component of the physiology impacting re-

Table 2

Comparison of mean escape response parameters for adult males and adult females of *Acartia tonsa* stimulated with photic (PH) or hydrodynamic (HY) stimuli

Parameter	Males			Females		
	PH	HY	<i>P</i>	PH	HY	<i>P</i>
Response latency (ms)	62.2	3.6	<0.001*	68.2	3.5	<0.001*
Initial turn (deg)	74.1	82.1	0.22	41.3	45.9	0.21
Jump speed (mm s <sup>-1</sup> )	293	213	0.09	256	188	<0.001*
Maximum speed (mm s <sup>-1</sup> )	494	432	0.001*	446	372	<0.001*
Max. acceleration (m s <sup>-2</sup> )	128	163	<0.001*	93	112	<0.001*
Number of thrusts	4.9	3.1	<0.001*	7.5	3.3	<0.001*
Minimum speed between thrusts (mm s <sup>-1</sup> )	147	166	0.272	130	133	0.889
Thrust duration (ms)	8.1	6.1	<0.001*	8.8	6.6	<0.001*
Jump duration (ms)	44.5	19.3	<0.001*	74.6	24.1	<0.001*
Distance jumped (mm)	8.6	4.2	<0.001*	12.5	4.6	<0.001*

Probability (*P*) based on the Mann-Whitney sum rank test. An asterisk \* indicates a significant difference ( $\alpha = 0.05$ ).

Data for responses to hydrodynamic stimuli are from Buskey *et al.* (2002).



sponse characteristics is the neural substrate for processing the sensory information and firing the motoneurons associated with effector muscle action. Although differences of 10–20 ms in the primary transduction processes could account for much of the observed difference in behavioral latencies in copepods, they are perhaps not enough to account for all. Both in *Drosophila* and several annelids, the system of giant fibers (axons) is involved in both photically and mechanically triggered reactions. The large diameter of these axons speeds the conduction of nerve impulses and is widely used throughout invertebrate groups in escape and startle reactions. Vertebrates utilize myelination of axons instead of large size to increase conduction speed. *Acartia* is known to possess giant mechanosensory fibers in its antennae (Yen *et al.*, 1992). It is not one of the myelinated species (Lenz *et al.*, 2000). It may be presumed to have similar giant fibers involved in escape reactions elsewhere in its nervous system, as do other copepods (Lowe, 1935; Park, 1966; Lenz *et al.*, 2000). However, the involvement of such elements is not yet certain in photic responses, and longer-latency pathways may be involved, as they are in the escape reactions of other organisms.

Several other aspects of photically triggered escape appeared different from those of the hydrodynamically triggered escape described by Buskey *et al.* (2002). Keeping in mind the intrinsic difficulties of inter-modality comparisons, we pooled the responses for copepods adapted to 0.3, 1, and 10  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  adaptation levels going to complete dark, which were not significantly different from one another in their escape parameters, to compare with the range of hydrodynamic stimulation of Buskey *et al.* (2002). In addition, adaptation temperature was slightly different for responses compared in the two studies (19 °C in this study vs. 22 °C in Buskey *et al.*, 2002), although there was no significant difference found in the response latencies of adult females of *A. tonsa* at 19 °C (Fig. 3) and 22 °C (Fig. 7) under the same light conditions. Maximum speeds in the photic responses were significantly higher for photically stimulated escape responses than for hydrodynamically stimulated responses in both male and female individuals of *A. tonsa* (Table 2). In contrast, maximum accelerations were significantly greater for hydrodynamically stimulated escape responses than for photically stimulated responses in both males and females. Photically stimulated responses were also clearly more extensive than the hydrodynamically stimulated responses, with significantly more thrusts, longer jump duration, and greater distance jumped (Table 2). Interestingly, the duration of individual thrusts within an escape response was significantly greater for photically stimulated escapes for both males and females. The pattern that emerges from the various comparisons is that both the triggering of the escape (including the orientation component, and the excitation that keeps the neural circuits active) and the characteristics of the motor output are modality-

specific. This calls into question the same central motor pattern generator being involved in both escape reactions. An increase in response vigor at the highest stimulus intensity was found in the present studies, but was not clearly apparent in our earlier work on mechanically evoked escape (Buskey *et al.*, 2002). However, other copepods do appear to adjust the vigor of response with the intensity of mechanical stimulus (Lenz and Hartline, 1999), so this feature, too, may be parallel between photic and mechanosensory systems. It would seem that the two sensory-motor systems have evolved semi-independently, each to protect the animal from a certain type of predatory threat. In doing so, they utilize common elements of motor pattern production and control; yet rather distinct circuitry that gives characteristics appropriate to each situation has been developed as well. When examined minutely, the escape circuitry of other organisms has often revealed a multiplicity of pathways at the motor as well as the sensory side (*e.g.*, Wine and Krasne, 1982; Eaton *et al.*, 2001). It remains to be seen whether such complications also occur in copepods.

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